

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Seung U. Kim  
SERIAL NO. : 09/887,145  
FILED : June 22, 2001  
FOR : "IMMORTALIZED HUMAN MICROGLIA CELL  
AND CONTINUOUS CELL LINE"  
EXAMINERS : Christopher J. Nichols & Gary Kunz  
GROUP ART UNIT : 1647  
ATTORNEY'S DOCKET NO. : UBC-002

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commission for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on: Sept. 19, 2003.

Attorney for applicant: David Prashken  
Signature: David Prashken  
Date: Sept. 19, 2003

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MARKED UP VERSION OF AMENDED SPECIFICATION SUBMITTED  
PURSUANT TO 37 C.F.R.1.121

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Sir:

Applicant, in fulfillment of and in accordance with the requirements of 37 C.R.F. 1.121(b)(1)(iii), hereby submits a marked up version of amendments to the Specification which appear at the following location:

Page 7,line 1;


Pages 27-29; and

Page 31, line 4.

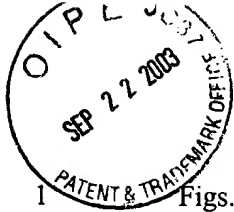
Respectfully submitted,

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Date: Sept. 19, 2003

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1 Figs. 6A-6E [B] are graphs showing the results of ELISA analyses for cytokines and  
2 chemokines released from normal human microglia and HMO6 immortalized human  
3 microglia cells; and

4 Fig. 7 is a photograph shows the cytogenetic analysis of HMO6 immortalized human  
5 microglia cells as the normal karyotype of human cells.

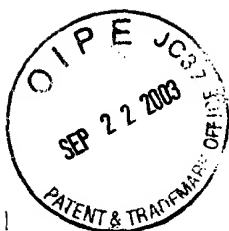
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7 DETAILED DESCRIPTION OF THE INVENTION

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9 The present invention is the establishment and characterization of several continuous  
10 cell lines of immortalized human microglia, labeled as HMO6, generated by transfection of  
11 embryonic (fetal) human microglia (HM) with a retroviral vector containing cDNA for the v-  
12 myc oncogene. The invention provides a phenotypic characterization of these immortalized  
13 human microglia; and discloses the expression of cytokines and chemokines following  
14 exposure to  $\beta$  amyloid peptides using HM and HMO6 cells. For a clearer understanding and  
15 better appreciation of the subject matter as a whole which comprises the present invention,  
16 the detailed description will be presented as separate sections.

17  
18 I. A Preferred Method For Producing Immortalized Human Microglia Cells And  
19 Continuous Cell Lines  
20

21 'Human microglial cell line, as used herein, means a human-derived cell line with  
22 microglial characteristics, including at least the specific antigens CD68 and CD11b. Also, as  
23 used herein, "non-fetal" refers to the fact that the progeny cells are expanded from



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Table E1: Sequences of PCR Primers

	<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u> (bp)
6	CD68 sense	AGATTCGAGTCATGTACACAACCCA [SEQ ID NO:1]	279
7	CD68 antisense	GGTGCTTGGAGATCTCGAAG [SEQ ID NO:2]	
9	P <sub>2Y1</sub> R sense	TGTGGTGTACCCCTCAAGTCCC [SEQ ID NO:3]	260
10	P <sub>2Y1</sub> R antisense	ATCCGTAACAGCCCAGAATCAGCA [SEQ ID NO:4]	
12	P <sub>2Y2</sub> R sense	CCAGGCCCCCGTGCTCTACTTTG [SEQ ID NO:5]	367
13	P <sub>2Y2</sub> R antisense	CATGTTGATGGCGTTGAGGGTGTG [SEQ ID NO:6]	
15	CXCR4 sense	TTCTACCCCAATGACTTGTG [SEQ ID NO:7]	206
16	CXCR4 antisense	ATGTAGTAAGGCAGCCAACA [SEQ ID NO:8]	
18	MIP-1 $\alpha$ sense	ACCATGGCTCTCTGCAACCA [SEQ ID NO:9]	393
19	MIP-1 $\alpha$ antisense	TTAAGAAGAGTCCACAGTG [SEQ ID NO:10]	
21	MIP-1 $\beta$ sense	CCTGCTGCTTTTCTTACACC [SEQ ID NO:11]	336
22	MIP-1 $\beta$ antisense	CACCTAATAACAATAACACGGC [SEQ ID NO:12]	
24	MCP-1 sense	ATAGCAGCCACCTTCATTCC [SEQ ID NO:13]	466
25	MCP-1 antisense	TTCCCAAGTCTCTGTATCT [SEQ ID NO:14]	
27	IL-1 $\beta$ sense	AAAAGCTTGGTGATGTCTGG [SEQ ID NO:15]	179
28	IL-1 $\beta$ antisense	TTTCAACACGCAGGACAGG [SEQ ID NO:16]	
30	IL-2 sense	ATGGTTGCTGTCTCATCAGC [SEQ ID NO:17]	301
31	IL-2 antisense	CTGGAGCATTTACTGCTGGA [SEQ ID NO:18]	
33	IL-3 sense	ATGAGCCGCTGCCCCGTCCTG [SEQ ID NO:19]	459
34	IL-3 antisense	AAGATCGCGAGGCTCAAAGTCGTCTGTTG [SEQ ID NO:20]	
36	IL-4 sense	GACACAAGTGCAATATCACC [SEQ ID NO:21]	337
37	IL-4 antisense	AAGTTTTCCAACGTACTCTG [SEQ ID NO:22]	
39	IL-5 sense	GAGGATGCTTCTGCATTTGAGTTTG [SEQ ID NO:23]	295
40	IL-5 antisense	GTCAATGTATTTCTTTATTAAGGACAAG [SEQ ID NO:24]	
42	IL-6 sense	GTGTGAAAGCAGCAAAGAGGC [SEQ ID NO:25]	159
43	IL-6 antisense	CTGGAGGTACTCTAGGTATAC [SEQ ID NO:26]	

Table E1: Sequences of PCR Primers (continued)

	<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u> (bp)
6	IL-7 sense	TGTTGAACTGCACTGGCCAG [SEQ ID NO:27]	484
7	IL-7 antisense	GCAACTGATACCTTACATGG [SEQ ID NO:28]	
9	IL-8 sense	ATGACTTCCAAGCTGGCCGTG [SEQ ID NO:29]	301
10	IL-8 antisense	TATGAATTCTCAGCCCTCTTCAAAA [SEQ ID NO:30]	
12	IL-9 sense	ATGCTTCTGGCCATGGTCCT [SEQ ID NO:31]	375
13	IL-9 antisense	TATCTTGCCTCTCATCCCTC [SEQ ID NO:32]	
15	IL-10 sense	AGATCTCCGAGATGCCTTCAGCAGA [SEQ ID NO:33]	194
16	IL-10 antisense	CCTTGATGTCTGGGTCTTGGTTCTC [SEQ ID NO:34]	
18	IL-11 sense	ACTGCTGCTGCTGAAGACTCGGCTGTGA [SEQ ID NO:35]	295
19	IL-11 antisense	ATGGGGAAGAGCCAGGGCAGAAGTCTGT [SEQ ID NO:36]	
21	IL-12 sense	TCACAAAGGAGGCGAGGTTCTAAGC [SEQ ID NO:37]	213
22	IL-12 antisense	CCTCTGCTGCTTTTGACACTGAATG [SEQ ID NO:38]	
24	IL-13 sense	ACCCAGAACCAGAAGGCTCCG [SEQ ID NO:39]	198
25	IL-13 antisense	TCAGTTGAACCGTCCCTGGCG [SEQ ID NO:40]	
27	IL-15 sense	AAACCCCTGCCATAGCCAACTCTT [SEQ ID NO:41]	202
28	IL-15 antisense	CTTCTGTTTTAGGGAGCCCTGCACT [SEQ ID NO:42]	
30	TNF- $\alpha$ sense	CAAAGTAGACCTGCCCAGAC [SEQ ID NO:43]	490
31	TNF- $\alpha$ antisense	GACCTCTCTCTAATCAGCCC [SEQ ID NO:44]	
33	NF-M sense	TGGGAAATGGCTCGTCATTT [SEQ ID NO:45]	333
34	NF-M antisense	CTTCATGGAAGCGGCCAATT [SEQ ID NO:46]	
36	MBP sense	ACACGGGCATCCTTGACTCCATCGG [SEQ ID NO:47]	510
37	MBP antisense	TCCGGAACCAGGTGGGTTTTAGCG [SEQ ID NO:48]	
39	GFAP sense	GCAGAGATGATGGAGCTCAATGACC [SEQ ID NO:49]	266
40	GFAP antisense	GTTTCATCCTGGAGCTTCTGCCTCA [SEQ ID NO:50]	
42	B7-2 sense	CTCTTTGTGATGGCCTTCCTG [SEQ ID NO:51]	464
43	B7-2 antisense	CTTAGGTTCTGGGTAACCGTG [SEQ ID NO:52]	

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Table E1: Sequences of PCR Primers (continued)

<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u>
G3PDH sense	CCATGTTTCGTCATGGGTGTGAACCA <u>[SEQ ID NO:53]</u>	251
G3PDH antisense	GCCAGTAGAGGCAGGGATGATGTTTC <u>[SEQ ID NO:54]</u>	

bp = base pairs.

## **Gene expression of cytokines and chemokines following AB treatment**

Gene expression of cytokines and chemokines in HM or HMO6.A1 cells was examined following a 6 hr treatment with or without 20  $\mu$ M of A $\beta$ <sub>25-35</sub> (NH<sub>2</sub>-GSNKGAIIGLM-COOH) [SED ID NO:55]. LPS at 100 ng/ml was used in microglial cultures since LPS is a potent activator of microglia [Gebicke-Jiacter *J. Neurosci.* 9: 187-194 (1989); Suzumura et al., *Brain Res.* 545: 301-306 (1991)].

## **ELISA analysis**

Production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  in normal human microglial cells or HMO6.A1 cells was determined in spent culture supernatants using ELISA kits specific for human TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  (R&D Systems, capable of detecting TNF- $\alpha$  at 4.4 pg/ml, IL-1 $\beta$  at 1 pg/ml, IL-6 at 0.70 pg/ml, IL-8 at 10 pg/ml and MIP-1 $\alpha$  at 10 pg/ml). At the end of each experiment, culture supernatants were collected, centrifuged, and stored at -70°C.

## **Experimental Series I: Isolation of human microglia cell lines**

Microglial-enriched populations were isolated from primary cultures of embryonic human telencephalon cells by virtue of differences in dish-adherent properties. The major differences are shown by Figs. 2A-2D respectively.

Fig. 2 as a whole shows the morphological appearance, and antigenic and functional tests of HM and HMO6.A1 cells. Fig. 2A is a phase contrast microscopy of HM; and Fig. 2B